

MARKED UP COPY OF AMENDED SPECIFICATION

Page 8, please replace the first paragraph of Example 1 with the following,

20 to 40µg of genomic DNA prepared from a bovine individual by simple extraction method was dissolved in 50µl×1 rTaq buffer (10mM Tris-HCl, 50mM KCl, 0.1% TritonX-100) containing 120µl of each dNTP, 1.5mM MgCl₂, 0.2µm of each primer and 2 units of recombinant Ta1 DNA elongation enzyme (rTaq)(TOYOBO). The solution was heated at 95°C for 5 minutes for denaturation, and then a cycle of reactions at 95°C for 50 seconds for denaturation, at 60°C for 50 seconds for annealing, and at 72°C for 50 seconds for elongation was repeated for 20 cycles. Then, elongation at 72°C for 2 minutes was performed. Primers used were those capable of specifically amplifying DRB3 gene exon 2 by PCR which encodes β1 domain of bovine MHC Class II DRβchain (BoLA-DRβ).

ERB3 : 5'- GGA ATT CCT CTC TCT GCA GCA CAT TTC C -3'

(The nucleotide sequence of ERB3 is shown as SEQ ID NO:10.)

HL031 : 5'- TTT AAA TTC GCG CTC ACC TCG CCG CT -3'

(The nucleotide sequence of HLO31 is shown as SEQ ID NO:11.)

Pages 8-9, please replace paragraphs (a), (b) and (B) of Example 1 with the following

(a) Forward primers specific for respective allele groups:

sp1: 5'- TGT AAA ACG ACG GCC AGT AGC ACA TTT CCT GCA GTA TC -3'

sp2: 5'- TGT AAA ACG ACG GCC AGT AGC ACA TTT CCT GGA GTA TTC TAA -3'

sp3: 5'- TGT AAA ACG ACG GCC AGT AGC ACA TTT CCT GGA GTA TTA -3'

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sp4: 5'- TGT AAA ACG ACG GCC AGT AGC ACA TTT CCT GGA GTA TTG -3'

sp5: 5'- TGT AAA ACG ACG GCC AGT CAC ATT TCC TGG AGT AGT -3'

sp6: 5'- TGT AAA ACG ACG GCC AGT GCA CAT TTC CTG GAG TAT C -3'

sp7: 5'- TGT AAA ACG ACG GCC AGT AGC ACA TTT CCT GGA GTA TA -3'

sp8: 5'- TGT AAA ACG ACG GCC AGT CAC ATT TCC TGG AGT ATT CTA C -3'

(Nucleotide sequences of sp1 to sp8 are shown as SEQ ID NOS: 1 to 8, respectively.)

(b) Reverse primer:

The following primer was designed as a primer capable of amplifying all alleles.

DRB3B: 5'- CAG GAA ACA GCT ATG ACC CGC CGC TGC ACA GTG AAA CTC -3'

(The nucleotide sequence of DRB3B is shown as SEQ ID NO:9.)

(B) PCR capable of amplifying all alleles:

25µl×1 GeneAmpR Gold Buffer (PE Biosystems), containing 120µl of each dNTP, 1.5mM MgCl₂, forward primer capable of amplifying 0.2µM of all allele groups, 0.2µl of reverse primer, 1 unit of AmpliTaq Gold™ DNA elongation enzyme and 1µl of the above obtained PCR products, was heated at 95°C for 10 minutes for denaturation as a pretreatment, and then a cycle of reactions at 95°C for 1minute for denaturation, at 64°C for 30 seconds for annealing, and at 72°C for 30 seconds for elongation was repeated for 20 cycles. Then, elongation at 72°C for 5 minutes was performed.

(a) Forward primer:

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DRB3ALL: 5'- TGT AAA ACG ACG GCC AGT ATT CCT CTC TCT GCA GCA CAT TTC CTG
-3'

(The nucleotide sequence of DRB3BALL is shown as SEQ ID NO:12.)

(b) Reverse primer:

DRB3B: 5'- CAG GAA ACA GCT ATG ACC CGC CGC TGC ACA GTG AAA CTC -3'

(The nucleotide sequence of DRB3B is shown as SEQ ID NO:9.)

MARKED UP COPY OF AMENDED CLAIMS

1. (Amended) A [primer set used in] method for amplifying [PCR for typing polymorphisms of] DNA[s] molecules encoding BoLA-DRB3.2 for typing alleles which comprises: performing PCR with [(1)] a reverse primer [capable of amplifying] which amplifies all alleles of BoLA-DRB3.2[:], and [(2)] a forward primer [capable of amplifying] which amplifies all alleles in any one of two or more groups of alleles of BoLA-DRB3.2 wherein each of said [group] groups comprises at least one allele, but [incapable of amplifying] which cannot amplify any allele(s) in the other group(s).

2. (Amended) The [primer set] method according to claim 1 wherein [a] the forward primer comprises a portion of a DNA sequence encoding an amino acid sequence of the first hypervariable region of BoLA-DRB3.2.

3. (Amended) The [primer set] method according to claim 1 [or claim 2] wherein 96 kinds of alleles of BoLA-DRB3.2 are classified into the two or more groups of alleles of BoLA-DRB3.2.

4. (Amended) The [primer set] method according to [any one of claims] claim 1 [to 3] wherein 96 kinds of alleles of BoLA-DRB3.2 are classified into 8 groups, and wherein the forward primer [is capable of amplifying] set amplifies all alleles in any one of said 8 groups but [incapable of amplifying] which cannot amplify any alleles in the other groups.

5. (Amended) The [primer set] method according to [any one of claims] claim 1 [to 4] wherein the forward primer comprises [a nucleotide sequence selected from the group consisting of

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the nucleotide sequences described in] any one of the sequences of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 and 8.

6. (Amended) The [primer set] method according to [any one of claims] claim 1 [to 5] wherein the reverse primer comprises [a nucleotide sequence described in] the sequence of SEQ ID NO: 9.

14. (Amended) A method for typing polymorphisms of DNA encoding BoLA-DRB3.2, which comprises [the steps of]:

(1) performing PCR using [the primer set according to any one of claims 1 to 6 a] bovine genomic DNA or a DNA fragment thereof as a template, a reverse primer which amplifies all alleles of BoLA-DRB3.2, and a forward primer which amplifies all alleles in any one of two or more groups of alleles of BoLA-DRB3.2 wherein each of said group comprises at least one allele, but which cannot amplify any allele(s) in the other group(s); and

(2) where at least two PCR products are amplified in [said step] (1), directly sequencing each of the amplified products, and where one PCR product is amplified in [said step] (1), sequencing the amplified product by using a primer set [capable of amplifying] which amplifies all alleles of BoLA-DRB3.2[,]; and

(3) [then] comparing resulting sequence(s) with known sequences of alleles and typing the polymorphisms.

15. (Amended) The method according to claim 14, wherein PCR is performed by using a primer set [capable of amplifying] which amplifies all alleles of BoLA-DRB3.2 using a bovine

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genomic DNA as a template, and then [said step] (1) is performed by using a resulting amplified product as a template.